RESEARCH ARTICLE

PHYTOCHEMICALS AND ANTIOXIDANT PROPERTIES OF THE LEAVES OF WILD GUAVA VARIETIES GROWN IN SRI LANKA

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ABSTRACT

Guava (*Psidium guajava* Linn.) is well-known throughout the world for its food, nutritional, and medicinal properties. Several guava cultivars/varieties are available in Sri Lanka, which can be classified as common, wild, or introduced. Though common guava has been extensively studied for its phytochemistry and pharmacology, only a few studies on wild varieties has been available so far. Therefore, this study focused on the investigation of phytochemical constituents and antioxidants capacity of two main wild guava varieties grown in Sri Lanka namely, *Psidium guajava* (cv. Getta-pera) and *Psidium guineense* (cv. Embul-pera). An Ultrasound-assisted-extraction technique was used to extract plant constituents, and water was used as the solvent. The phytochemicals were qualitatively and quantitatively analyzed using standard methods whereas the antioxidant capacity was determined using the DPPH and FRAP assays. Phytochemical screening revealed that both varieties contain most of the important phytochemicals. Though both showed higher anti-oxidant activity, Embul-pera had the highest in both the FRAP and DPPH assays, with 612.69±0.50 mg Trolox Eq/g and IC_{50} value of 191.69±0.25 ppm respectively. The highest level of all quantified phytochemicals, particularly polyphenolic content (327.87±0.23 mg GAE/g extract) was found in Embul-pera. As a conclusion, two wild guava varieties considered in the study contain a diverse phytochemical profile and higher antioxidant properties similarity to the common guava. It can be recommended that Getta-pera" and "Embul-pera" are excellent alternatives to be used in functional foods and nutraceuticals preparation and hence to promote the cultivation as economic plants.

Keywords: *Antioxidants, embul-pera, getta-pera, phytochemicals, wild guava*

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1. INTRODUCTION

The genus *Psidium* (Family: Myrtaceae) is native to the Tropical and Subtropical Americas and contains approximately 92 species worldwide. Brazil is a major *Psidium* diversity hotspot, with approximately 60 species, 47 of which are endemic [1], [2]. The most important species in this genus are guava (*Psidium* spp.) [1]. *Psidium guajava*, *Psidium cattleyanum*, and *Psidium guineense* are the most important commercial species in fruits production and as a source for chemical compounds in the pharmaceutical industry [2]. Different parts of the plant are used in traditional medicine to treat various ailments including wounds, ulcers, bronchitis, eye sores, bowels, diarrhoea, and cholera. Phytochemical studies on different parts of the plant by many researchers has reported diverse phytochemicals with their chemical structures [3]. Pharmacology of commonly available guava has been reported to high extent [4].

Sri Lanka is abundant in guava cultivars/varieties, *i.e.* commonly consumed, wild and introduced varieties. Many cultivars are available in common-guava, *P. guajava*, such as pink, red, and white flesh fruits, and small, middle, and large size fruits; wild verities are getta-pera (a cultivars of *P. guajava*), apple-guava (*P. pomiferum)*, embul-pera/sour-guava (Wild, *P. guineense*) and strawberry-guava (*P. cattleyanum*); introduced varieties are kanthi, pubudu, horana red, horana white, costorican, etc. [5], [6]. The common guava, particularly pink and white - fleshed middle size fruit, is available throughout the country whereas strawberry guava and sour-guava are mostly found in the southern province of Sri Lanka and introduced varieties can primarily be obtained from the Fruit Crops Research and Development Centre (FCRDC), Horana, Sri Lanka [5]. Notably, both getta-pera and common guava are cultivars of *P. guajava*. Despite the fact that both getta-pera and common guava belong to the same genus and species, they can be distinguished by their texture, flavour, and seed yield. Getta-pera has a gritty surface, too many seeds, and a sour taste, whereas common guava has a smooth texture, is delightfully scented, is less seeds, and has a pleasant taste [6]. Though Sri Lanka is a home for a diverse range of guavas, only the common guava has attracted interest by the public [5] and less attention particularly on wild varieties for food and healthcare purposes. Therefore, research on Sri Lankan guava varieties needs to be strengthened for popularizing many verities among the public and to promote them as agricultural crops, and transform them into functional foods.

With this understanding, this study was led to screen and quantify the phytochemicals and evaluate the antioxidant capacity of aqueous extracts of leaves from two wild varieties of guava, getta-pera (*P. guajava*) and embul-pera (*P. guineense*). Non-conventional extraction techniques *i.e.* ultrasound-assisted extraction, was used to make efficient extraction via reducing extraction time, solvent consumption and increase of extraction yield.

2. MATERIAL AND METHODS

2.1 Sample collection

Getta-pera (*P. guajava*, Figure 01) and embul-pera (*P. guineense*, Figure 02) leaves were collected in Matara, Sri Lanka (Sample size-03) and authenticated in the Peradeniya Botanical Garden, Sri Lanka (The authenticated voucher specimens' numbers: *P. guajava*-AHEAD/DOR 05/G1 and *P. guineense*-AHEAD/DOR 05/G3). Healthy leaves were washed in tap water, then distilled water, and air-dried for a day. Dried leaves were ground in a mixture grinder to be used
in the extraction process.

Figure 01: Getta-pera (*P. guajava*)

Figure 02: Embul-pera (*P. guineense*)

2.2 Extraction

Ultrasound-assisted extraction was used to extract bioactive compounds from leaves. The finally grounded leaves of each plant (100.00 g) were sonicated for one hour at 30-35 ºC in an ultrasound-assisted extractor (ROCKER Ultrasonic cleaner, Model: SONER 202H) with distilled water (500 ml) [7]. The extracts were filtered through cotton plugs, followed by filter paper (Whatman No-01) and after removing of water under freeze drying (Model: FE-10-MR, S/No: FD 2020062222), resulted crude extracts were stored at 4ºC until further use.

2.3 Phytochemical qualitative analysis

Qualitative tests for phytochemicals such as polyphenol, flavonoid, tannin, saponin, terpenoid, alkaloid, coumarin, glycoside, anthocyanin, phytosterol, quinones, betacyanin, and chalcones, were performed in triplicates for each aqueous extract of leaves using standard procedures described in the literature [8], [9].

2.4 Phytochemical quantification

The aqueous extract (0.10 g) was dissolved in a small amount of DMSO and diluted with methanol (100 ml) to make a 1000 ppm concentration, which was then used for spectrophotometric quantification of polyphenolics, tannins, flavonoids, terpenoids and saponins as given below.

2.4.1 Total Phenolic content (TPC) and Total Tannin contents (TTC)

The TPC and TTC were estimated using a slightly modified Folin and Ciocalteu method [10], [11]. In brief, a mixture of FC reagent (2.5 ml) was added to the prepared sample extract (0.5 ml) and allowed to stand for 5 minutes. After 30 minutes, 2mL of Na_2CO_3 solution (7.5 percent w/v) was added and incubated and then the absorbance was measured at 765 nm. TPC was calculated using a gallic acid standard curve (0–100 ppm), and TPC of aqueous extracts was expressed in gallic acid equivalents (mg GAE/g extract). TTC was calculated using a tannic acid standard curve (0–100 ppm), and TTC of aqueous extracts was expressed in tannic acid equivalents (mg TAE/g extract).

2.4.2 Total Flavonoid contents (TFC)

TFC was estimated using a slightly modified spectrophotometric method described in [12], [13]. In brief, prepared sample extract (1.0 ml) was mixed with 2 percent $AlCl_3$ solution (0.5 ml) and 0.5 ml of distilled water and allowed to stand for 10 minutes after vigorously shaking the mixture. At 425 nm, the absorbance was measured. TFC was calculated using a quercetin standard curve (0–22 ppm), and TFC of aqueous extracts was expressed in Quercetin equivalents (mg QE/g extract).

2.4.3 Terpenoid contents (TC)

TC was estimated using a slightly modified spectrophotometric method [10]. In brief, 1 ml of 5 percent aqueous phosphomolybdic acid solution was added to 1 ml of sample extract, followed by 1 ml of the con. H_2SO_4 was gradually added. The mixture was thoroughly mixed and left for 30 minutes before being diluted to 5 ml with MeOH. The absorbance was recorded at 700 nm.TC was calculated using a Linalool standard curve (0–2.4 mM), and TC of aqueous extracts was expressed in Linalool equivalents (mg LE/g extract).

2.4.4 Saponin contents (SC)

SC was determined using a spectrophotometric method described in [14], [15]. In brief, 8 percent vanillin (1.0 ml) was mixed with 1 ml of prepared sample extract, then placed in an ice-water bath, followed by 8 ml of 77 % H_2SO_4 (v/v). After shaking, the test tube was placed at 60° C in an oven for 30 minutes. The solution was cooled in an ice-water bath for 10 minutes before being brought to RT for UV analysis and then the absorbance was measured at 540 nm. The SC of the extracts was expressed in Saponin equivalents (mg SE/g extract) (0-500 ppm) using a Saponin standard curve

2.4.5 Alkaloid contents (AC)

AC was determined using a spectrophotometric method described in [16], [17]. A portion of the aqueous extract was dissolved in the HCl solution (2N) before being filtered. One

milliliter of this supernatant was transferred to a separatory funnel, and washed with 10 mL of chloroform (3 times). The pH of this prepared sample was adjusted to neutral using 0.1 N NaOH. The resultant solution was then mixed with prepared BCG solution (5.0 ml) and freshly prepared phosphate buffer solution (pH 4.7, 5.0 ml). It was dynamically shaken, and the complex mixture was re-extracted with $CHCl_3$ (1, 2, 3, and 4 ml). The extracted complex mixture was then poured into a volumetric flask (10 ml), and it was diluted and adjusted with $CHCl_3$. The complex's absorbance in $CHCl_3$ was measured at 470 nm. The AC of aqueous extracts was expressed in Atropine equivalents (mg AE/g extract) (0-10 ppm) using an Atropine standard curve.

2.5 Antioxidant analysis

2.5.1 DPPH Radical Scavenging Assay

With some modifications, the free radical (FR) scavenging activity of guava leaves aqueous extracts were determined using the standard protocol described in the literature [18],[19]. The DPPH solution in MeOH (0.06 mM, 3.9 mL) was carefully mixed with 100 µL of various concentrations of guava leaves aqueous extracts. After 30 minutes in the dark, the absorbance at 517 nm was measured. The IC_{50} value for free radical scavenging activity was calculated using a percentage of scavenging effect vs. concentration plot. As a control, ascorbic acid and Trolox were used.

2.5.2 Ferric Reducing Antioxidant Power Assay (FRAP Assay)

The FRAP value of the aqueous extracts was determined using a standard method described in the literature [20],[21],[22]. About 3 ml of freshly prepared FRAP reagent [300 mM acetate buffer (pH-3.6): 10 mM TPTZ (in 40 mM HCl): 20 mM $FeCl_3$ in a ratio 10:1:1) was mixed with 100 µL of diluted sample. After 30 minutes of incubation at 37 °C, the absorbance at 593 nm was measured. For calibration, a Trolox solution (0–100 ppm) was used.

2.6 Statistical Analysis

Analysis of variance (ANOVA), T-test (LSD) (LSD-Least Significant Difference), and non-parametric statistics Cochran's Q test was used to analyze the data and make comparisons. The statistical analysis was carried out using SAS, R-studio, and Excel. The data were presented as means and standard deviations.

3. RESULTS AND DISCUSSION

3.1 Extraction

Ultrasound-assisted extraction (non-conventional) was used in this study to obtain extract rich in bioactive compounds. The extraction yields for Getta-pera and Embul-pera are 4.3735 ± 0.1878 % and 3.0593 ± 0.4151% respectively. Getta-pera yielded more than Embul-pera, according to the results. The qualitative and quantitative studies of bioactive

Table 01: Statistically analyzed phytochemical screening results of aqueous extracts of leaves of two guava varieties (P: Present, A: Absent).

Phytochemicals	Test method	Wild guava varieties	
		Getta-pera	Embul-pera
Alkaloids	Mayer's Test	P	P
	Wagner's Test	P	P
	Dragendroff's Test	P	P
Glycosides	Keller-kilani Test	P	P
	Modified Borntrager's Test	A	A
	Legal's Test	P	P
Flavonoids	Alkaline reagent Test	P	P
	Shinoda Test/ Mg turning Test	P	P
	Lead acetate Test	P	P
	$AlCl_3$ Test	P	P
	NH_4OH Test	P	P
Saponins	Froth Test	P	P
	Olive Oil Test	P	P
Tannins	Bramer's Test	P	P
	Lead Acetate Test	P	P
Terpenoids	Salkowski's Test	P	P
	Liebermann- Burchardt Test	P	P
	Copper acetate Test	P	P
Polyphenols	Ferric Chloride Test	P	P
Coumarins	UV light Test	A	A
	NaOH Test	P	P
Anthocyanins	HCl & NH3 Test	A	A
Chalcones	NaOH Test	A	A
Phytosterol	Salkowski's Test	P	P
Betacyanin	NaOH Test	P	P
Quinones	H_2SO_4 Test	P	P

compounds derived from plant materials are heavily reliant on the choice of an appropriate extraction method. Over the last 50 years, various extraction procedures have been developed to extract bioactive compounds from plants. The non-conventional extraction method, ultra-sound assisted extraction, used here is an efficient method at a low-cost [23].

3.2 Phytochemical qualitative analysis

Table-01 lists the phytochemicals found in aqueous extracts of Getta-pera and Embul-pera. It revealed the presence of highly important secondary metabolites in Getta-pera and Embul-pera leaves, including alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, polyphenol, coumarins, phytosterol, betacyanin, and quinones. It was found that anthocyanins and chalcones were absent in aqueous extracts of both varieties. Non-parametric analysis of Cochran's Q test was used to statistically determine the presence and absence of phytochemical availability in each plant sample. Non-parametric analysis Cochran's Q test demonstrated that these phytochemicals are present in both aqueous extracts of Getta-pera and Embul-pera leaves.

This finding lends credence to the outstanding pharmacological activities associated with guava leaves and the use of guava leaves in traditional medicine.

3.3 Phytochemical quantitative analysis

Quantitative analysis of polyphenol, tannin, flavonoid, terpenoid, saponin, and alkaloid, revealed that both Getta-pera and Embul-pera contain varying amounts in the leaves shown in Table-02. Interestingly, TPC, TTC, TFC, TC, SC, and AC levels were higher in Embul-pera (327.87 ± 0.23 mg GAE/g extract, 324.58 ± 0.23 mg TAE/g extract, 36.98 ± 0.03 mg QE/g extract, 10.44 ± 0.01 mM LE/g extract, 505.76 ± 1.65 mg SE/g extract, and 2.36 ± 0.22 mg AE/g extract, respectively) than Getta-pera. These new findings were compared to earlier findings based on a methanolic extract of common guava (*P. guajava)* [4]. The quantities of TPC (479.29 ± 2.16 mg GAE/g extract), TTC (437.54 ± 0.57 mg TAE/g extract), and TC (19.72 ± 0.06 mM LE/g extract) were higher in methanolic extracts of common guava compared to the aqueous extracts of Getta-pera and Embul-pera leaves. In contrast, TFC was higher in aqueous extracts of two wild varieties than the methanolic extract of common guava (28.15 ± 0.09 mg QE/g extract) [4].

T-test (LSD) statistical analysis was performed on each phytochemical quantification data such as polyphenol, flavonoid, tannin, terpenoid, saponin, and alkaloid. According to the T-test (LSD), the quantities of all phytochemicals were significantly ($\alpha = 0.05$) different between the two guava varieties, except for AC, as shown in Figures-03

Table 02: Quantitative Phytochemical Analysis and results of FRAP assay of aqueous extracts of Getta-pera and Embul-pera leaves. Values represent mean ± standard deviation of triplicate sample.

Test Name	Wild guava varieties	
	Getta-pera	**Embul-pera**
Phenolic content (mg GAE/g extract)	261.47 ± 0.23	327.87 ± 0.23
Flavonoid content (mg QE/g extract)	33.13 ± 0.07	36.98 ± 0.03
Tannin content (mg TAE/g extract)	258.84 ± 0.23	324.58 ± 0.23
Terpene content (mM LE/g extract)	9.30 ± 0.03	10.44 ± 0.01
Alkaloid content (mg AE/g extract)	2.15 ± 0.19	2.36 ± 0.22
Saponin content (mg SE/g extract)	462.43 ± 2.86	505.76 ± 1.65
FRAP Assay (mg Trolox Eq/g extract)	441.05 ± 0.88	612.69 ± 0.50

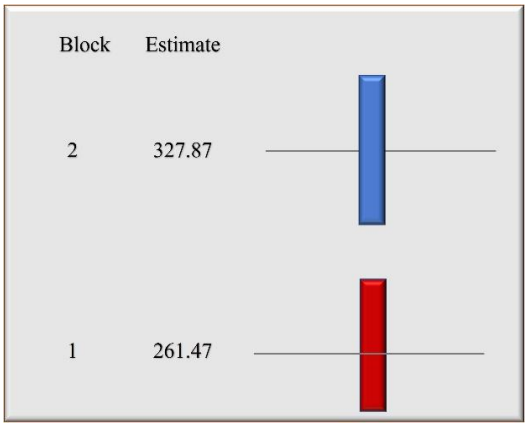

Figure 03: T-test (LSD) for quantification of TPC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

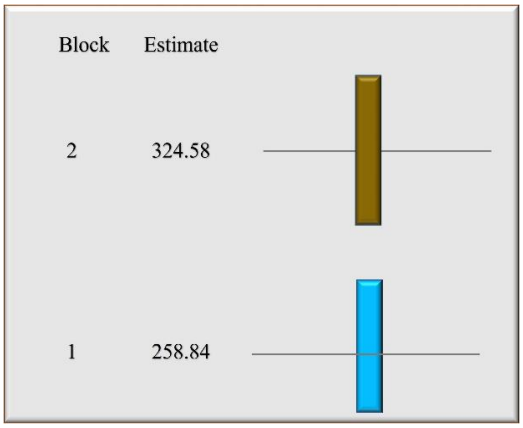

Figure 04: T-test (LSD) for quantification of TTC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly

In particular, quantified phytochemicals except the alkaloid were present in different levels in aqueous extracts of Getta-pera and Embul-pera leaves, showing higher in Embul-pera extract than Getta-pera extract at a 5% significant level. In contrast, AC was present at the same level in both at a 5% significant level, and the amount was comparatively lower than that of other quantified phytochemicals.

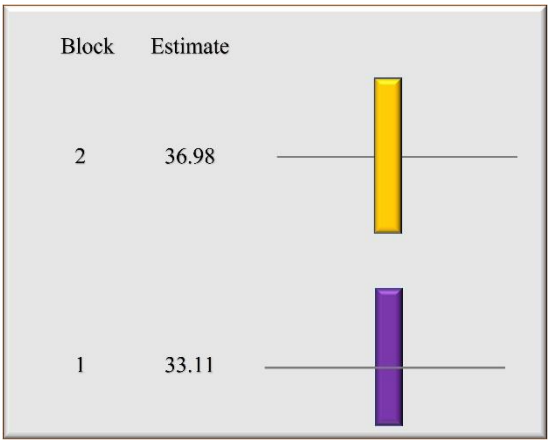

Figure 05: T-test (LSD) for quantification of TFC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

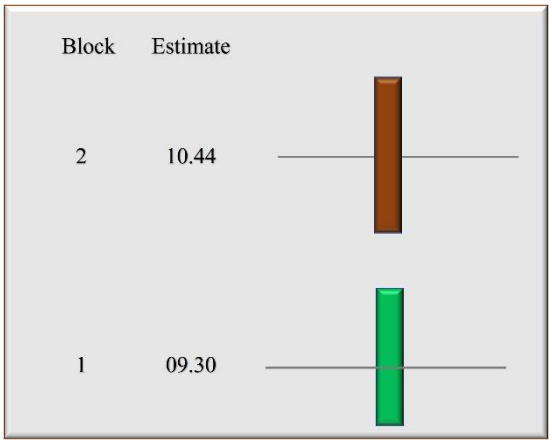

Figure 06: T-test (LSD) for quantification of TC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

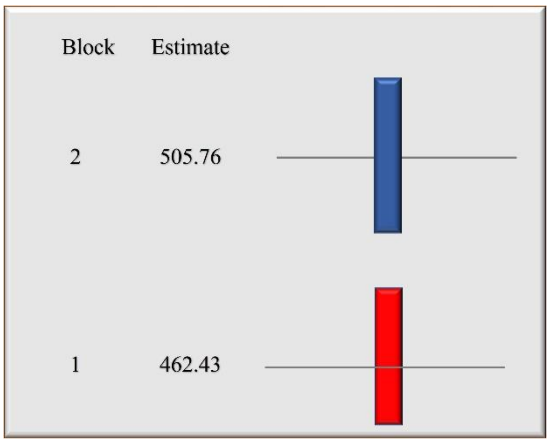

Figure 07: T-test (LSD) for quantification of SC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

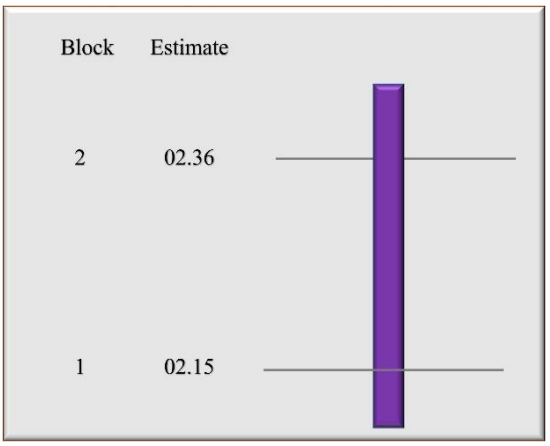

Figure 08: T-test (LSD) for quantification of AC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

3.4 Antioxidant analysis

3.4.1 DPPH Assay

The DPPH assay results are expressed as IC_{50} values (concentration required to inhibit 50 % of the oxidative reaction). Figure-09 depicts the results of the DPPH assay, and Trolox and Ascorbic acid were used as standards to compare with aqueous extracts of guava varieties' leaves. According to the findings, Embul-pera had the highest radical scavenging activity (IC_{50} value: 191.69 ± 0.25 ppm). These results obtained were compared to past studies on the antioxidant activity of methanolic leaf extracts of guava cultivars' [4]. Getta-pera, Embul-pera and common guava had IC_{50} values of 232.02 ±

0.42, 204.14 ± 0.15, and 192.89 ± 0.07 ppm, respectively in methanolic extracts in our previous study [4]. It showed that aqueous extracts had better antioxidant capacity than methanolic extracts of the same.

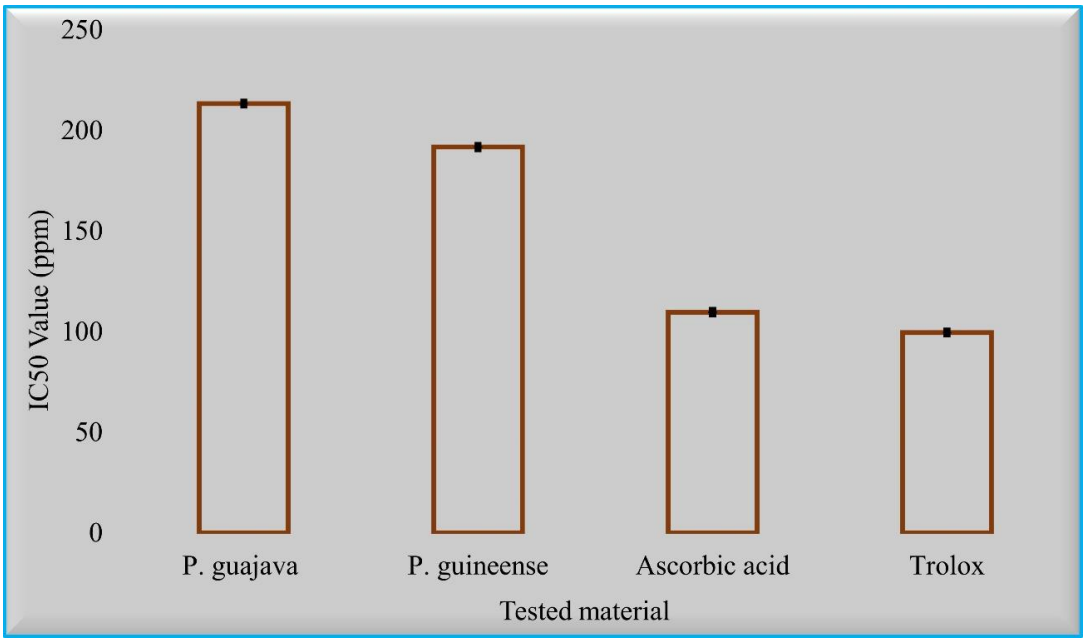

Figure 09: DPPH assay data of aqueous extracts of two wild guava varieties and standards (Error bars indicate the standard deviation).

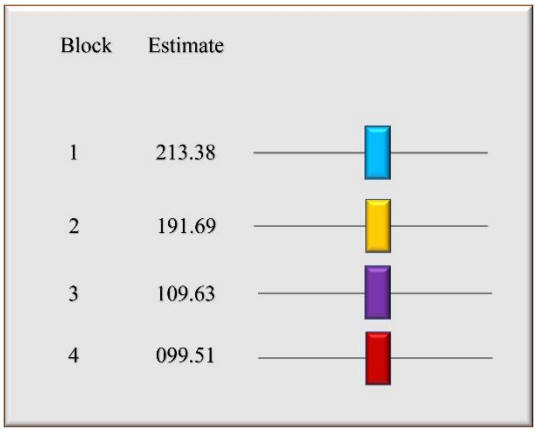

Figure 10: T-test (LSD) for DPPH assay of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, 3: Ascorbic acid, 4: Trolox, means covered by the same bar are not significantly different).

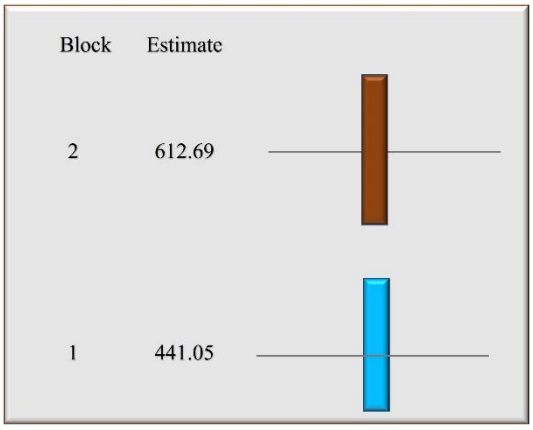

Figure 11: T-test (LSD) for FRAP assay of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

T-test (LSD) of DPPH radical scavenging activity perfectly revealed that both aqueous extracts of Getta-pera and Embul-pera leaves and both standards Ascorbic acid and Trolox are statistically significant difference at 5% significant level, as shown in Figure-

10. This means that the leaves of Getta-pera and Embul-pera each have their own distinct feature in terms of radical scavenging activity. However, of the two guava varieties tested, Embul-pera demonstrated the highest radical scavenging activity.

3.4.2 FRAP Assay

Table-02 displays the Ferric reducing power of aqueous extracts of Getta-pera and Embul-pera leaves. Both extracts exhibit reducing power, but at different extents. The aqueous extract of Embul-pera leaves had the highest reducing power (612.69 ± 0.50 mg Trolox Eq/g) out of both. Furthermore, statistical analysis; T-test (LSD) revealed that there were no significant similarities in ferric reducing power between both aqueous extracts at the 5% significant level, as shown in Figure-11. Furthermore, the results of this study were compared to the data previously published by us for methanolic extracts of the same [4]. The reducing power of methanolic leaf extracts of Getta-pera, Embul-pera, and Common guava is 677.23 ± 2.66, 640.12 ± 3.01, and 722.44 ± 6.58 mg Trolox Eq/g, according to that [4]. All guava species, including common guava, have a higher reducing power than aqueous extracts at a 5% significant level whereas the aqueous extracts of Getta-pera and Embul-pera have acceptable reducing power. The current study found that wild varieties are an excellent source of antioxidants and other phytochemicals.

Natural antioxidants are to reduce the risk of numerous diseases, including atherosclerosis, reperfusion injury, cataractogenesis, rheumatoid arthritis, inflammatory disorders, cancer, the aging process etc. Natural antioxidants secure the human body from harmful free radicals, thereby preventing oxidative stress and the other diseases that it causes [24]. According to our findings, both the leaves of Getta-pera and Embul-pera aqueous extracts are a source of natural antioxidants that could lead to the development of functional foods, nutraceuticals, and to discover novel drugs [24]. As outcomes from the current study, it can be suggested that widely distributed two wild guava varieties in Sri Lanka, namely Getta-pera and Embul-pera, could be used to prepare functional foods, nutraceuticals, or phytomedicine.

CONCLUSION

Wild guava varieties, namely Getta-pera and Embul-pera contain a wide range of important phytochemicals and high antioxidant capacity. As outcome of the study, these two wild guava leaves could be used in preparing of functional foods and nutraceuticals to be used in health enhancement purposes and could be promoted as marketable varieties.

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REFERENCES

[1] Santos M. A. C, Rêgo M. M, Queiróz M. A, Caproni D. T. R, Dietrich O. H. S, Santos A. F, Rocha D. I, Batista D. S, Otoni W. C, (2020). In vitro growth performance of *Psidium guajava* and *P. guineense* plantlets as affected by culture medium formulations. *Vegetos*, **33**: 435-445.

[2] Marques A. M, Tuler A. C, Carvalho C. R, Carrijo T.T, Ferreira M. F. S, Clarindo W. R, (2016). Refinement of the karyological aspects of *Psidium guineense* (Swartz, 1788): a comparison with *Psidium guajava* (Linnaeus, 1753). *Comp Cytogenet*, **10**(1): 117-128.

[3] Begum S, Hassan S. I, Ali S. N, Siddiqui B. S, (2004). Chemical constituents from the leaves of *Psidium guajava. Natural Product Research*, **18**(2): 135 –140.

[4] Díaz-de-Cerio E, Verardo V, Gómez-Caravaca A. M, Fernández-Gutiérrez A, Segura-Carretero A, (2017). Health Effects of *Psidium guajava* L. Leaves: An Overview of the Last Decade. *International Journal of molecular Sciences*, **18**(4): 897.

[5] Kokilananthan S, Bulugahapitiya V. P, Gangabadagea C.S, Manawadua H, (2020). Comparative account on antioxidant properties, proximate and phytochemical compositions of seven guava varieties grown in Sri Lanka. *Journal of Agriculture and Value Addition*, **3**(2): 1–16.

[6] Menzel, C. M, (1985). Guava: An exotic fruit with potential in Queensland. *Queensland Agricultural Journal*, **111**(2): 93-98.

[7] Musa K. H, Abdullah A, Jusoh K, Subramaniam V. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. *Food Analytical Methods*, **4**: 100-107.

[8] Gayathri V, Kiruba D, (2014). Preliminary phytochemical analysis of leaf powder extracts of *Psidium guajava* L. *International Journal of Pharmacognosy and Phytochemical Research*, **6**(2): 332-334.

[9] Wadood A, Ghufran M, Jamal S. B, Naeem M, Khan A, Ghaffar R, (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry and Analytical Biochemistry*, **2**(4): 1-4.

[10] Ekwueme F, Nwodo O, Joshua P, Nkwocha C, Eluka P, (2015). Qualitative and quantitative phytochemical screening of the aqueous leaf extract of Senna mimosoides: Its effect in in vivo leukocyte mobilization induced by inflammatory stimulus. *International Journal of Current Microbiology and Applied Sciences*, **4**(5): 1176-1188.

[11] Prabhavathi R, Prasad M, Jayaramu M, (2016). Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. *Advances in Applied Science Research*, **7**(4): 11-17.

[12] Fernandes M, Azzolini A, Martinez M, Souza C, Lucisano-Valim Y, Oliveira W, (2014). Assessment of antioxidant activity of spray dried extracts of *Psidium guajava* leaves by DPPH and chemiluminescence inhibition in human neutrophils. *BioMed Research International*, **2014**: 1-10.

[13] Pękal A, Pyrzynska K, (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods*, **7**: 1776-1782.

[14] Ahmed H. O. A, Wang C, (2015). Determination of tea saponin in Camellia seed oil with UV and HPLC analysis. *World Journal of Engineering and Technology*, **3**(4): 30-37.

[15] Du M, Guo S, Zhang J, Hu L, Li M, (2018). Quantitative analysis method of the tea saponin. *Open Journal of Forestry*, **8**(1): 61-67.

[16] Ajanal M, Gundkalle M.B, Nayak S.U, (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. *Ancient science of life*, **31**(4): 198-201.

[17] Shamsa F, Monsef H, Ghamooghi R, Verdian Rizi M, (2007). Spectrophotometric determination of total alkaloids in *Peganum harmala* L. using bromocresol green. *Research Journal of Phytochemistry*, **1**: 79-82.

[18] Blois M, (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, **181**: 1199-1200.

[19] Brand-Williams W, Cuvelier M. E, Berset C, (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, **28**(1): 25-30.

[20] Biglari F, AlKarkhi A.F, Easa A.M, (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chemistry*, **107**(4): 1636-1641.

[21] Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L, (2005). Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochimica et Biophysica Acta*, **1721**(1-3): 174-184.

[22] Gliszczyńska-Świgło A, (2006). Antioxidant activity of water-soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. *Food chemistry*, **96**(1): 131-136.

[23] Azmir J, Zaidul I.S.M, Rahman M, Sharif K, Mohamed A, Sahena F, Jahurul M, Ghafoor K, Norulaini N, Omar A, (2013). Techniques for extraction of bioactive compounds from plant materials: *A review. Journal of food engineering*, **117**(4): 426-436.

[24] Barros L, Cabrita L, Boas M. V, Carvalho A. M, Ferreira I. C, (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry*, **127**(4): 1600-1608.